A Century of SEPARATION SCIENCE

edited by
Haleem J. Issaq
SAIC Frederick
NCI-Frederick Cancer Research
and Development Center
Frederick, Maryland

MARCEL DEKKER, INC. NEW YORK • BASEL

25

The Impact of Analytical Supercritical Fluid Extraction and Supercritical Fluid Chromatography on Separation Science

Jerry W. King

National Center for Agricultural Utilization Research, Agricultural Research Service/USDA, Peoria, Illinois

I. INTRODUCTION AND HISTORICAL PERSPECTIVE

The role of supercritical fluid media, or "critical" fluid technology (which also includes the near critical state) in analytical separation science developed from several diverse sources, originating as a hybrid technique of gas chromatography in the mid-1960s. The most often cited origins of supercritical phenomena are from phase equilibria studies or geochemical processes conducted at high pressures and temperatures [1]. Interest in this particular "intermediate state" of matter also found early application in petroleum recovery [2], which was followed by pioneering research in the late 1960s on applying supercritical fluids for the extraction of solutes (e.g., caffeine) and as a versatile reaction medium (polymerizations, hydrogenations). In parallel with this latter trend was the observation that chromatographic retention parameters were effected by column pressure when utilizing nonideal fluids such as carbon dioxide as eluents. This gave rise to a different form of gas chromatography called "dense gas chromatography" [3], which permitted intractable solutes of limited volatility to be solubilized in the mobile phases and separated as a function of eluent pressure or density.

Without doubt the most recognizable advocate for this new separation technique was J. Calvin Giddings of the University of Utah [4], from whom the author received his initial training and introduction to the field. These early academic-based studies in dense gas chromatography (GC) were frustrating due to the lack of commercial equipment as well as problems in sample

---

Names are necessary to report factually on available data; however the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the products to the exclusion of others that may also be suitable.

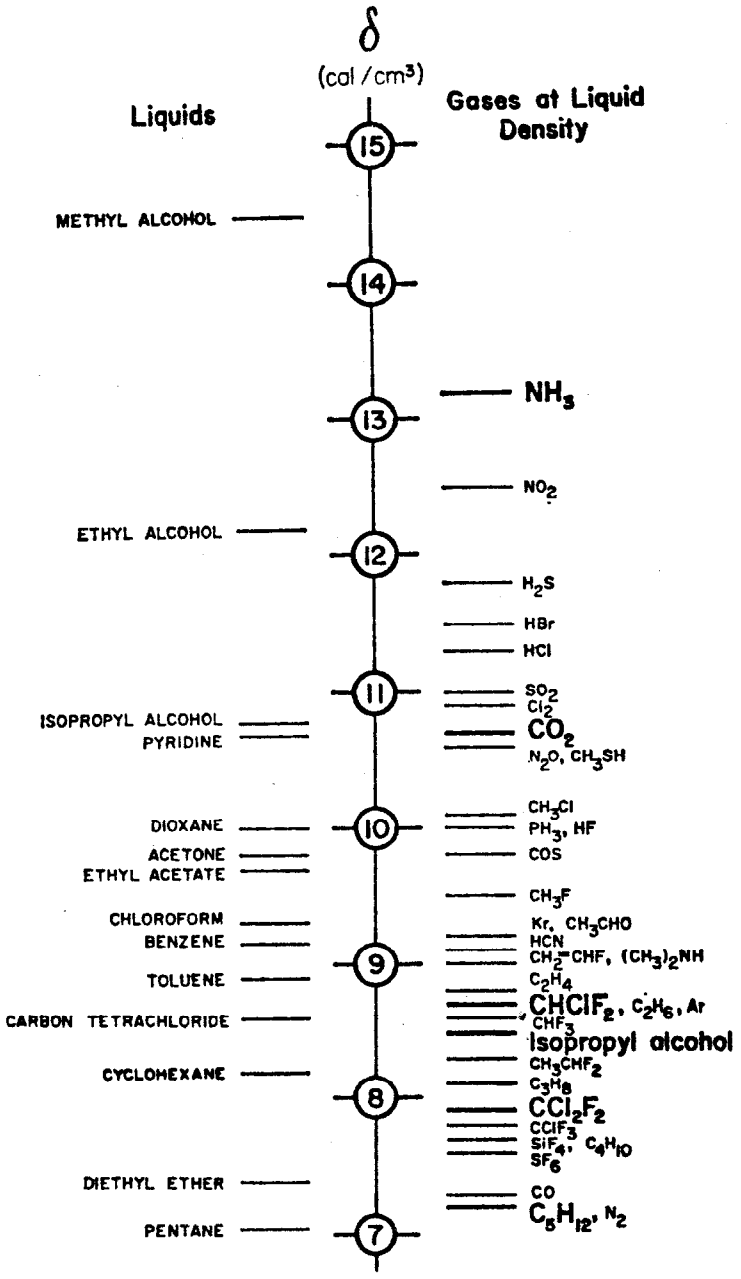

Figure 1 Solubility parameters of compressed gases at liquid-like densities compared to solubility parameters of liquids in their condensed state. (Reproduced with permission of American Association for the Advancement of Science.)

introduction and solute detection under such extreme conditions. It was not until the mid-1970s that high-performance liquid chromatography (HPLC) injection techniques were merged with bonded phase column technology [5] to produce a viable analytical chromatograph, which was eventually commercialized in a open tubular column format in the early 1980s.

One of the seminal concepts to come out of the early dense GC studies was the correlation of gas (supercritical fluid) solvent power with that exhibited by various liquids as characterized by the solubility parameter concept [6]. As illustrated in Fig. 1, dense gases can attain solubility parameters at a high level of compression that are equivalent to those of liquid solvents; however, the ability to mechanically adjust the solvent power and hence the selectivity of these fluids by regulating the applied pressure is a key feature that makes supercritical fluid chromatography (SFC) and extraction (SFE) unique. Figure 2 illustrates this principle nicely, where the solubility parameter of the fluid is shown as a function of pressure at several different temperatures. Over the given temperature range, it is apparent that CO_2 attains a higher solubility parameter than either nitrogen or helium. This is due to the convient critical temperature for CO_2, 31°C, and the nonideal fluid properties exhibited by CO_2 over this particular temperature range. Figure 2 also shows that no matter the temperature, CO_2 experiences a considerable increase in solvent power close to its critical pressure of 72 atm (1060 psi).

Although solubility parameter theory was only intended to provide an approximate estimate of a compressed fluid's solubility properties, it has been refined by this author [7,8] and others [9,10] to provide excellent quantitative prediction and correlations for many solute types in SFE and SFC. This basic concept obtained from initial SFC studies has been profusely cited in the literature up to the beginning of the new millennium and has served as a guide in both analytical as well as engineering applications of supercritical fluids.

Retention patterns of solutes in supercritical fluids are pressure or fluid density dependent, as indicated in Fig. 3, for even a simple solute such as benzene when using supercritical carbon dioxide (SC-CO_2) as an eluent. Chromatographic elution parameters such as capacity factors or retention volumes can vary over several magnitudes as a supercritical fluid eluent's pressure is

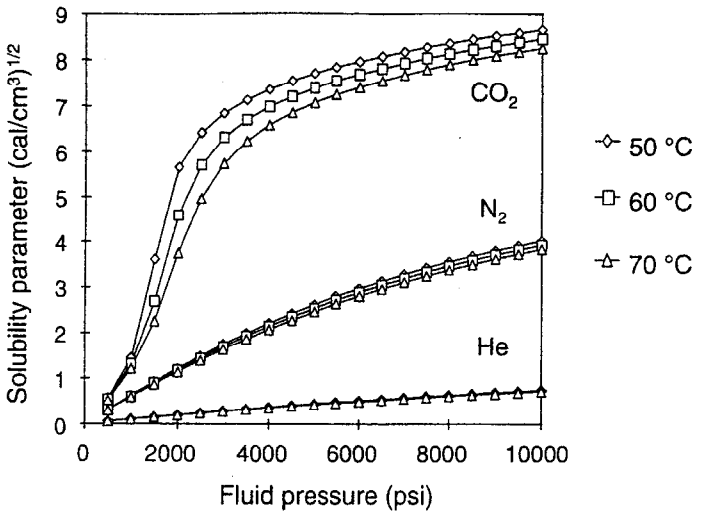

Figure 2 Solubility parameters for helium, nitrogen, and carbon dioxide versus fluid pressure. (From Ref. 35.)

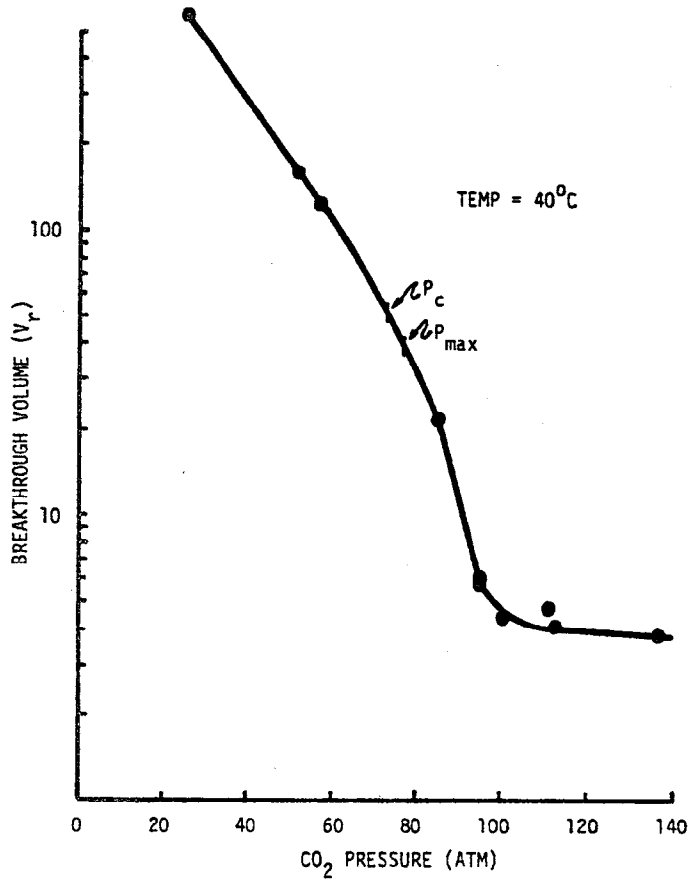

Figure 3 Retention volume for benzene versus carbon dioxide pressure on a styrene/divinylbenzene column. (From Ref. 11.)

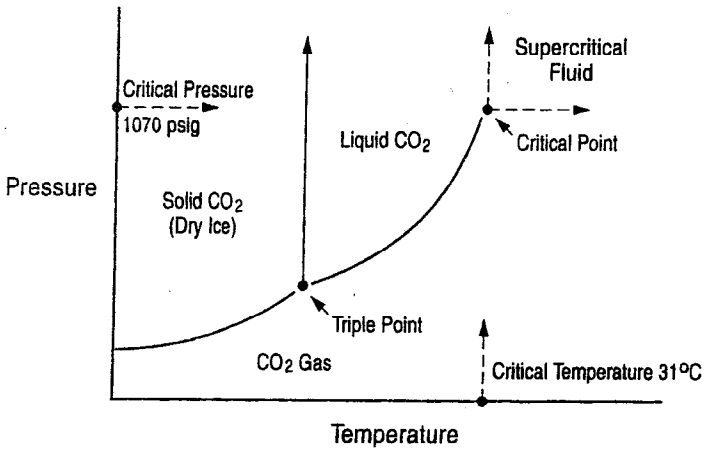

Figure 4 Phase diagram for carbon dioxide. (From Ref. 48.)

changed; a reflection of solute's solubility enhancement in the supercritical fluid or the fluid's interaction with the stationary phase in the chromatographic column [11,12]. This serves as the basis for separating solutes when chromatography is conducted in either the isobaric, isconfertic, or pressure programmed modes. Pressure programming has become by far the most popular mode when performing SFC, although modern instrumentation also allows temperature and flow programming to be enacted during the analysis. The addition of organic solvents to the mobile phase became a requisite with the introduction of packed column SFC [13], for enhancing solute solubility in the mobile phase, and for adjustment of an analyte's retention durng SFC analysis. These addition components to the mobile phase are called cosolvents or modifiers and can only be dissolved in a finite amount in the supercritical fluid at a given pressure and temperature to avoid a two-phase eluent system.

Differences in opinion have arisen over the years as to the need to preserve the fidelity to a true one-phase eluent system in SFC. As show in Fig. 4, the conventional supercritical state

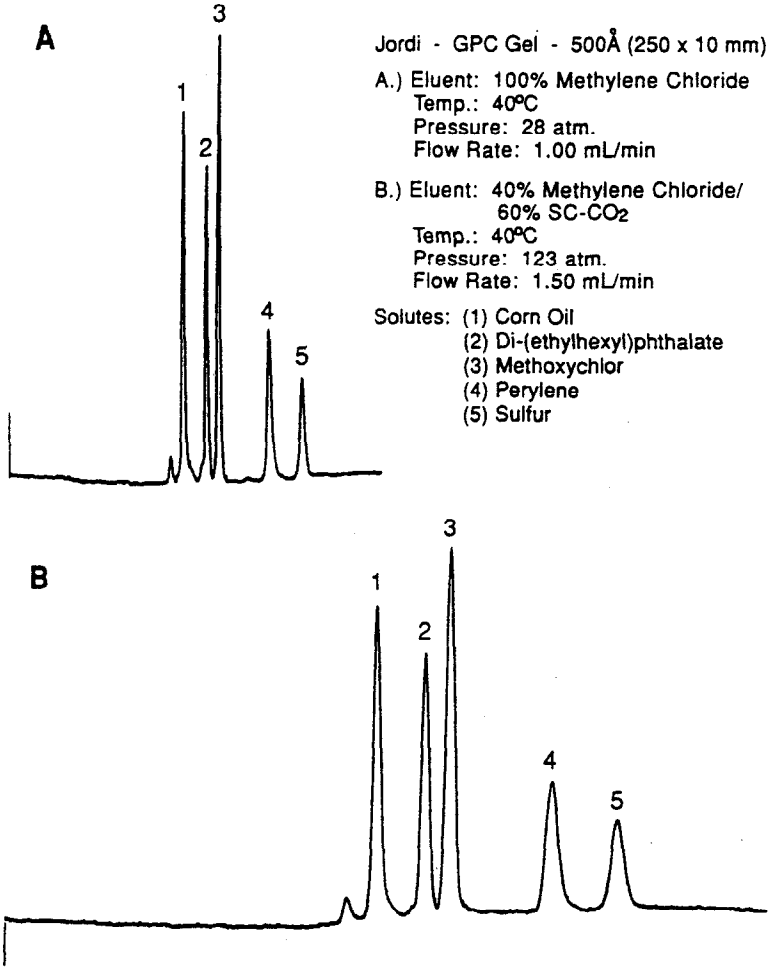

Figure 5 Comparison of conventional liquid-based SEC separation vs. SEC separation using liquid eluent and SC-CO_2.

for fluids such as CO_2 was often defined as that region above the fluid's critical temperature and pressure, respectively. However successful chromatographic separations have been attained in the liquid CO_2 region and by using pressures and temperatures below the critical point of eluent. This arbitrary boundary (indicated by the dashed lines in Fig. 4) for the supercritical state has been noted by Chester [14] and has perhaps retarded the use of experimental conditions outside the conventional definition of the supercritical state. Recently, solvating gas chromatography [15] has been reported under conditions outside the arbitrary boundary of the supercritical fluid state, although such conditions were also utilized by Sie and coworkers [16] over 30 years ago. King and coworkers [17] have demonstrated that two-phase eluent systems, in which one of the mobile phase components is a fluid under supercritical conditions and one a liquid, can yield useful analytical separations. This is demonstrated in Fig. 5, where the addition of the weaker eluent component, SC-CO_2, to methylene chloride does not detract but actually enhances the separation of a five-component mixture. Similar beneficial effects have been attained using a supercritical component in conjunction with a conventional liquid solvent by Olesik [18], called enhanced fluidity chromatography.

II. THE MATURATION OF SFC AND ITS IMPACT ON ANALYTICAL SEPARATION SCIENCE

SFC matured as a viable analytical technique throughout the 1980s and 1990s as documented in well-known texts by Lee and Markides [19], Berger [20], and Anton and Berger [21]. Curiously, it was the technique of packed column SFC that first became available commercially through the efforts of the Hewlett Packard Company [22]; although this development was followed by the introduction of capillary instrumentation in the United States by the Lee Scientific Company. This latter mode of SFC became quite popular and served as the basis for several well-known symposia that exhibited the potential of the technique throughout the 1980s.

The use of capillary SFC was not without its pitfalls, being limited to a practical extent by very narrow-bore columns having limited solute capacity and the use of the flame ionization detector. Nonetheless, when performed properly, capillary SFC utilizing SC-CO_2 could be the method of choice when analyzing many complex mixtures. As a trace analysis technique (ppm and ppb), capillary SFC as well as packed column SFC was limited by the analyst's ability to couple many of the hetero-element detectors (ECD, NPD, etc.) to achieve stable, reproducible analysis. Some success has been achieved using either ultraviolet (UV) or evaporative light scattering detection (ELSD), particularly when coupled with packed column SFC. The efforts of Taylor and colleagues [23] in attempting to utilize a variety of detectors in SFC are worth noting.

The true worth of a separation technque is found in its routine application to everyday analysis problems. As noted in Table 1, capillary SFC excells in several areas of analysis, most notably in the environmental analysis of petroleum derivatives, the characterization of oligomer mixtures of natural and synthetic polymers, and class separations of species having significant molecular weight differences. This has resulted in several standard methods, including the extension of simulated distillation curves over that which can be achieved using gas chromatography. The method also permits class separations of lipid species [24] and has found routine application in preference to GC and HPLC in the author's laboratory. As illustrated in Fig. 6, relative short analysis times (45 min) can be achieved using capillary SFC to resolve the components found in a deodorizer distillate sample. The elution order in this case, as with many SFC separations, is based on molecular weight differences in the solutes, thereby allowing large separation factors to be achieved between such lipid species as free fatty acids, mono-, di-, and triglycerides, and,

Table 1 Successful applications of SFC

Hydrocarbon and PAH mixtures
Petroleum compound class separations
Oligomeric mixture fractionation
Lipid class separations
Essential oils
Simulated distillation chromatography
Separation of enantiomers
Surfactants
Explosives
Pesticides

in some cases, phospholipids [25]. Packed column SFC lagged behind capillary SFC in application for many years, but in the late 1990s it has undergone a renaissance due to the development of more versatile instrumentation and niche applications in both pharmaceutical and petrochemical analysis. The high-resolution factors attained by using packed column SFC for the separation of enantiomers [26] has seen SFC establish itself as a competitive technique with HPLC, and class separations of petrochemical mixtures has become an official method. The ability to use SFC in conjunction with flame ionization detection has also provided the pharmaceutical analyst

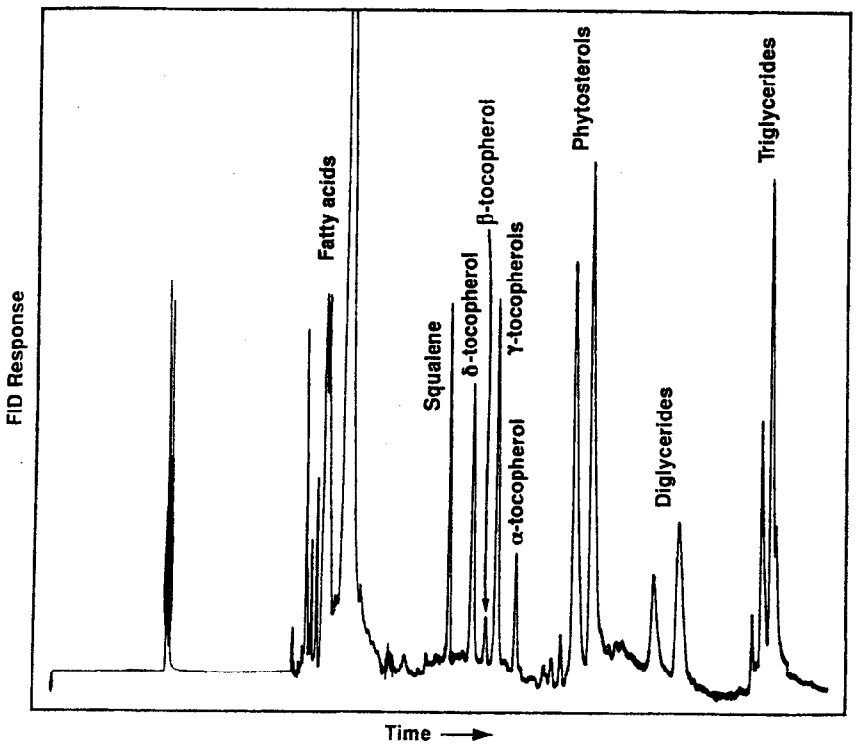

Figure 6 Separation of lipid components in deodorizer distillate by capillary SFC. (From Ref. 25.)

Table 2 Applications of SFC in Industrial Analysis

Elimination of sample preparation
Deformulation of commercial products
Raw material specifications
Monitoring of reaction products and kinetics
Support of supercritical fluid research
Analysis of minor components
Characterization of small samples (with SFE)
Analysis of thermally labile analytes
Determination of physicochemical properties

with a complementary method to HPLC analysis, particularly for the detection of nonchromaphoric contaminents in drug formulations and raw materials.

SFC has proven itself directly applicable in industrial analysis, as noted in Table 2. The ability to elute both the components of interest as well as interfering components using pressure or density programming can reduce or eliminate sample preparation prior to analysis. Programming of the SFC mobile phase can also be utilized for deformulating specific types of products into their individual constituents, as demonstrated in Fig. 7 for the capillary SFC resolution of a lipstick formulation [27]. SFC has also proven facile in the author's laboratory in support of supercritical fluid-based research projects, i.e., the monitoring of enzyme-initiated reactions in SC-CO_2 and the characterization of extracts obtained via SFE with SC-CO_2.

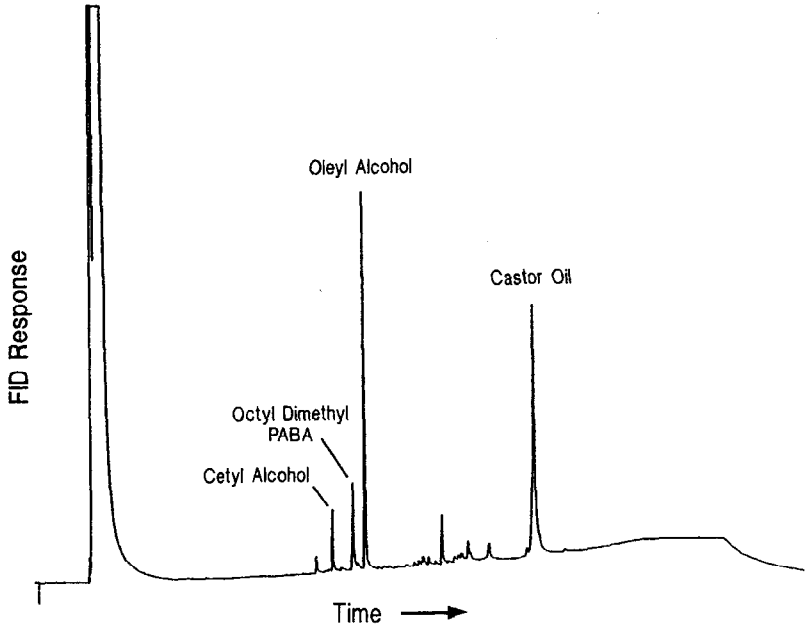

Figure 7 Separation of components in a quencher lipstick formulation. (From Ref. 27.)

Table 3 Determination of Physicochemical Properties by SFC

Diffusion coefficients
Sorption isotherms
Phase distribution constants
Solubility measurements
Critical loci
Partial molar volumes
Virial coefficients

The measurement of physicochemical properties by SFC is an often overlooked application of the technique. Table 3 lists some of these properties that have been determined by SFC. The diffusion coefficients of dissolved solutes in dense fluids were first measured as early as 1968 by the chromatographic band broadening method, but more diffusion coefficient data need to be collected on solutes that are feasible industrially to extract with CO_2. Quasi-equilibrium properties, such as phase distribution constants or solubility measurements, can be rapidly achieved using SFC [28], particularly on a relative basis when the value of a reference compund is known beforehand. Retention parameter shifts under certain conditions will yield solute partial molar volumes or second interaction virial coefficients for solute-fluid interactions. So-called threshold pressures, first approximated by the author in enhanced migration studies with Giddings and Myers [4], can be measured by SFC, as indicated in Fig. 8 for the pesticide malathion in SC-CO_2. This critical locus was established by measuring the first appearance of a peak for malathion on a nitrogen-phosphorus detector downstream from the SFC column, which is

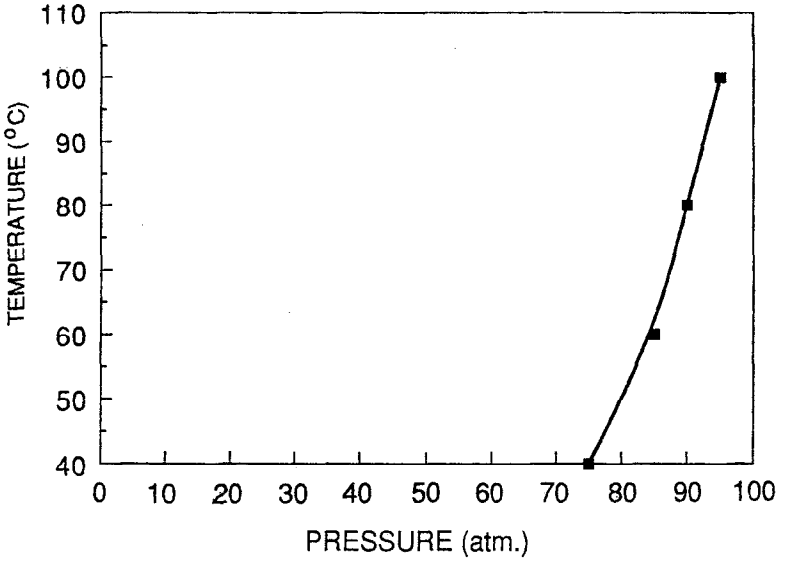

Figure 8 Threshold pressure for malathion in SC-CO_2 as a function of temperature and pressure as determined by SFC with a nitrogen/phosphorus detector.

Table 4 Detection Limits of Techniques Used to Assess Threshold Pressures in Supercritical Fluids

Detection principle	Sensitivity (g)
Gravimetric	10^{-3}
Infrared spectroscopy	10^{-7}
Ultraviolet spectroscopy	10^{-9}
Mass spectrometry	10^{-9}
Flame ionization	10^{-10}
TLC visualization	10^{-12}

commensurate with the solvation of the pesticide by SC-CO_2. However, as noted by the author [29], threshold pressures can depend on the measurement technique as shown in Table 4, with chromatographic-based methods being particularly sensitive to the dissolved solute's concentration in the fluid phase due to the detection methods employed.

Analytical-scale SFC, even at low resolution, can provide useful data for optimizing critical fluid based processes. The ease or difficulty in extracting solutes from a particular sample matrix can be assessed by using elution pulse chromatography [30], and minature sorbent-filled columns can likewise be used in the SFC mode to select sorbents for the preparative/production scale SFC of numerous compounds. The author [31] and others [32] have used this method to optimize SFC conditions for the enrichment of tocopherols or phospholipids from agriculturally derived products or to predict breakthrough of volatiles and nonvolatile compounds from sorbents. An outstanding example of this experimental philosophy was the development of appropriate conditions for the enrichment and fractionation of ethyl esters of fish oils by Lembke [33] using an 200 × 4 mm, i.d. analytical column. This process was eventually scaled up to production plant size in Tarragona, Spain.

III. THE DEVELOPMENT OF ANALYTICAL SFE

Analytical SFE was developed after SFC, although the basis of the technique was available in the engineering literature in the late 1960s. Pioneering studies by Stahl and coworkers [34] actually demonstrated the potential of SFE for both processing and analytical purposes utilizing thin layer chromatography. Analytical chromatographers using SFC initially foresaw that SFE would be a complementary on-line technique to SFC, applicable to relatively small samples that would yield extracts commensurate with the operation of either capillary or packed column SFC. However, in the early 1990s, concern about the use and disposal of organic solvents in the analytical laboratory became a focal point for government agencies such as the Environmental Protection Agency (EPA) and the National Institute for Occupational Safety and Health (NIOSH), and related agencies such as the U.S. Department of Agriculture (USDA) and Food and Drug Administration (FDA) responded in kind to demonstrate their compliance with these environmental and worker safety concerns.

This format provided our introduction into analytical SFE rather than the practice of SFC. Our laboratory had already been involved in developing processing concepts using supercritical fluids for close to a decade, hence we could incorporate concepts used in process SFE into the design of an optimal analytical SFE system for toxicant and nutritional analysis of foods and agricultural products. This was the beginning of a synergestic approach in our critical fluid research, which

allowed developments in processing, or vice versa in analytical, to be transferred between these related fields [35]. Research for a related USDA agency, the Food Safety and Inspection Service (FSIS), set the guidelines for the development of our off-line SFE methodology. This required that analytical SFE be capable of replacing several liters of organic solvent used per sample in FSIS's traditional methodology, and that sample sizes of the order of 25–50 g be extracted.

Initial studies focused on the quantitative extraction of lipid (fat) from meat samples since the analytes of interest (pesticides) were contained in the peritoneal fat phase of the meat matrix. The moisture content of these sample matrices proved to be inhibitory in allowing contact between the SC-CO_2 and the lipid phase, and this was eventually solved by dehydrating the meat matrix initially and later simplified by the use of "extraction enhancer" called Hydromatrix (Varian, Harbor City, CA). This was the beginning of a number of original contributions of our research group in applying SFE to food and natural product analysis. Other research teams in parallel were beginning to apply SFE to the analysis of environmental samples [36], polymers [37], and drugs [38], each area characterized by unique problems that had to be solved. For example, SFE of soil samples was difficult due to sample matrix-analyte interactions, but despite these difficulties at least three EPA-approved methods were generated in a relatively short time during the early 1990s.

On-line analytical SFE was to become the dominant technique as opposed to on-line SFE due to simplicity of operation and compatibility with existing methodology. On-line methods, while inherently more sensitive, required considerable skill on the analyst's part and could not be adjusted easily for diverse applications. Figure 9 is a schematic of our generic laboratory SFE units at NCAUR, which have served as templates for commercial instrumentation (e.g., Spe-ed Unit, Applied Separations, Inc., Allentown, PA). Either liquid-cooled piston pumps or booster pumps/compressors are commonly used to deliver the CO_2 into an extraction cell, which is placed in a heated oven or zone to assure supercritical extraction conditions. Pressure and flow are regulated by a narrow orifice device placed downstream from the cell. This can be a tubular restrictor, micrometering valve, backpressure regulator, or an automatically controlled valve to compensate for changes in fluid flow and hence backpressure. Extracts are collected in one of several devices after the extracting fluid stream is decompressed, and these may consist of an empty or solvent-filled vial, a sorbent-filled tube or cartridge, or a subambient cooled device. The importance of adequate extract collection cannot be overemphasized, since excellent analyte recoveries via SFE can be reduced due to poor collection efficiency [39].

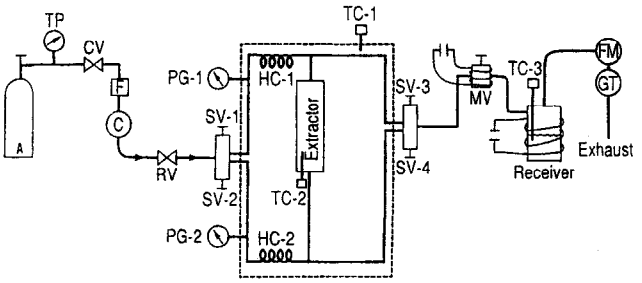

Figure 9 General laboratory SFE unit. A = CO_2 cylinder; TP = cylinder pressure gauge; CV = check valve; F = filter; C = air-driven gas booster compressor; RV = relief valve; SV = on/off switching valve; PG = pressure gauge; HC = equilibration coil; TC = thermocouple; MV = micrometering valve; FM = flow meter; GT = gas totalizer.

The need to process multiple samples was recognized early on by this investigator, and it seemed prudent to design an extractor that mimicked existing equipment utilized by analytical chemists in their laboratories (e.g., Soxhlet extractor). This gave rise to the development of a parallel sample extractor, which was co-developed with Marvin Hopper of the FDA laboratory in Lexena, Kansas [40]. This gave rise to several commercial offerings embracing this principle, perhaps the most succesful being the FA-100 Fat Analyzer produced by Leco, Inc. (St. Joseph, MI). The original NCAUR prototype consisted of six tubular extraction vessles, approximately 2 feet in length, ½ in. internal diameter, that provided a 100 mL cell volume for large samples. Sample size in the early days of analytical SFE was quite controversial, but our design was guided by the amounts specified in traditional protocols and the need to have a representative sample when extracting agricultural commodities with localized toxicant contamination (e.g., aflatoxins). The analytical SFE industry eventually offered an assortment of sample sizes, but a 10 mL capacity was considered adequate for most needs.

Analytical SFE was considered a complex technique to apply by some due to the large number of experimental parameters that had to be considered in optimizing an analysis. Table 5 shows most of experimental parameters that impact on conducting successful SFE. Many of these parameters have been commented on previously, but those unique to SFE and not other sample preparation procedures are probably pressure and flow rate. All of the other parameters have their analogs in competing methodology, although the vernacular may be somewhat different. Analytical SFE faced stiff competition from other alternative sample preparation procedures that minimized solvent use, such as solid phase microfiber extraction (SPME), accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE). However, the extraction specificity of SFE through the minipulation of pressure and temperature is perhaps what intrigued analytical chemists most about the technique.

Indeed, SFE can perform crude fractionation relative to chromatography by changing the fluid density, but it is rare to obtain a "clean" extract unless the sample matrix is insoluble in the supercritical fluid and the compounds to be isolated from each other differ substantially in their physicochemical properties. For example, the separation of fat from a foodstuff or contaminants in a soil sample can be handled quite adequately by SFE. On the other hand, the isolation of pesticides from a food sample that contains appreciable quantities of fat or water may be more problematic. This is the type of problem that faced our research team and hence was the

Table 5 Experimental Variables in Analytical SFE

Pressure
Temperature
Flow rate
Extraction time
Collection technique
Sample size
Homogeneity of sample
Choice of supercritical fluid
Choice of modifier
Amount of modifier
System leaks
Sample matrix effects
System contamination

Table 6 Options for Integrating Sample Clean-up with SFE

Fluid density–based fractonation
Supercritical fluid adsorption chromatography
Integration of selective adsorbents
Alternative fluids to carbon dioxide
On-line SFE/chromatography methods
Inverse SFE
SF-modified size exclusion chromatography (SEC)
Use of binary gas mixtures

basis for initiating research to integrate sample clean-up techniques into the basic SFE scheme. Table 6 lists some of the possibilities for sample clean-up integration with SFE and the systematic approach we took in solving the problem. In some cases, a judicious choice of extraction fluid density may provide an extract that is perfectly acceptable for analysis without the need for further clean-up. More common in SFE practice is to use a sorbent, either in the cell or after decompression, to further fractionate the extract. These sorbents tend to be normal phase chromatography adsorbents; therefore, SFC may be useful as a screening tool to choose the optimal sorbent for clean-up of the extract under SFE conditions. Another sorbent-based method invented by the author is "inverse" SFE, where a sorbent is incorporated into the extraction cell to isolate the target analyte of interest under SFE conditions, while interfering compounds are removed by the extraction fluid. This concept has been exploited in pesticide residue analysis as well as fractionation of pharmaceutical-based preparations [41].

Although SC-CO_2 reigns supreme as the prime extraction fluid in analytical SFE, other fluids can sometimes be put to advantage. For example, fluoroform, HCF_3, can be used rather than SC-CO_2, resulting in an extract with 100 times less fat than that obtained with SC-CO_2 under identical extraction conditions. Likewise, one can blend binary mixtures of fluids in their supercritical state to achieve a homogeneous extraction fluid that is more specific for analytes such as pesticide residues [42]. Recalling Fig. 2, it is apparent that using a supercritical fluid with a lower critical temperature than CO_2, such as nitrogen, the solubility parameter of the extracting fluid will be less than when using neat SC-CO_2. This is one of the reasons that a 70 mol% CO_2/30 mol% N_2 mixture will give extracts containing less than 5 mg of fat while assuring complete recovery of pesticides at the ppm level [42].

It is not possible to cover all of the aspects of analytical SFE and its optimization in this short review and memoir. The reader is referred to several primer texts [43,44] in the field and more advanced treatises or reviews [45,46] for further information. However, it is instructive to look at several of the applications of analytical SFE that demonstrate how the technique is utilized and its areas of applicability.

IV. APPLICATIONS OF ANALYTICAL SFE

Table 7 lists areas of application in which analytical SFE has been applied successfully. Within each generic class of compounds, there are certain compounds or subclasses that have not been extracted successfully using SC-CO_2, such as the β-lactam drugs. The results obtained with SFE are also somewhat matrix dependent, therefore, certain pesticides that are extracted successfully from foods may be more problematic, or require a change in conditions, for removal from soils. This is also true when using other sample preparation methods. Overall, pesticides as a com-

Table 7 Application Areas for Analytical SFE

Pesticides
Petroleum products
Environmental samples
Fat and lipid analysis
Drugs and antibiotics
Polymer oligomers/additives
Metal analysis
Volatiles and flavors

pound class extract well using SC-CO_2 or SC-CO_2/modifier mixtures, and the reader should consult the review by Lehotay [47] on this subject.

One of major successes of analytical SFE is in the extraction of fat and similar lipid substances from foods. In this regard, the author's research group has been a major contributor to this field, with over 15 publications concerned with various aspects of fat determination. This interest did not occur by accident, since our success was based on research conducted for the extraction of oils from seed matrices [48]. Studies in our laboratory clearly showed that extraction of fat or oil triglycerides could best be accomplished at pressures approaching 10,000 psi and temperatures in the range of 70–80°C, where their solubility was maximized (see Fig. 10). This set the upper operational limit with respect to pressure for most commercial instrumentation. Our group also was successful in integrating new official methodology for total and speciated fat analysis, as mandated by the Nutritional Labeling & Education Act (NLEA), with SFE. This included the development of enzyme-catalyzed reaction under supercritical fluid conditions to form fatty acid methyl esters (FAME) required in the NLEA analysis [49]. It should be noted that lower pressures and temperatures are frequently used for selectively extracting other lipid

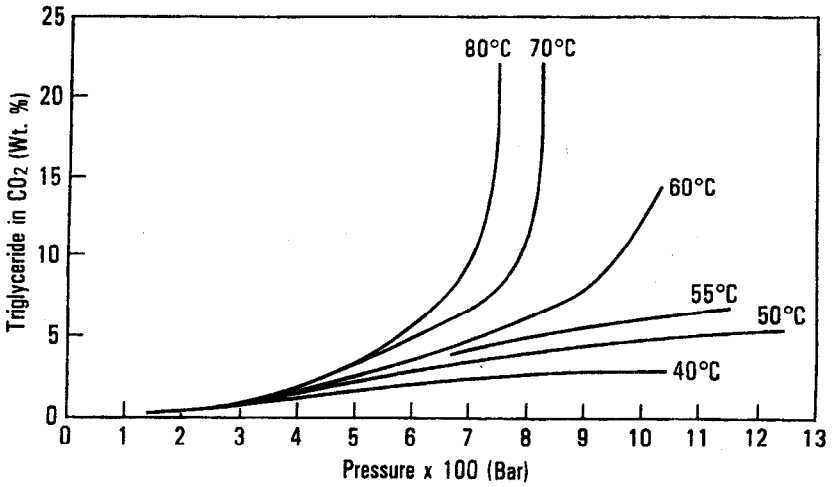

Figure 10 Effect of changing pressure and/or temperature on the solubility of triglycerides in SC-CO_2. (From Ref. 48.)

species such as cholesterol and fat-soluble vitamins, while phospholipids require the addition of a cosolvent (ethanol) for successful SFE. Such solubility findings were eventually incorporated into the development of a standard method for determining the oil content of vegetable seeds [50].

Analytical SFE has also experienced success when applied to the analysis of drugs in foods, biological matrices, and pharmaceutical preparations. In this field of application it is not unusual to employ a small quantity of cosolvent dissolved in SC-CO_2 to accelerate the extraction of the drug from the sample matrix. As noted previous, early success using SFE was recorded in the environmental analysis field, particularly in the extraction of organochlorine pesticides and dioxins, polynuclear aromatic hydrocarbons, and total petroleum hydrocarbons (TPH), which are all registered as official EPA methodology. The TPH method utilized SC-CO_2 as a replacement for a banned fluorocarbon previously used in running the method. Additional uses for analytical SFE are currently being developed, particularly in the areas of natural product analysis, extraction of metals, and as a more benign method for characterizing the volatile content of food and flavor components [51].

It is worth noting several examples from the author's work to illustrate some of the benefits of analytical SFE. For example, numerous "coupled" techniques have been generated using SFE

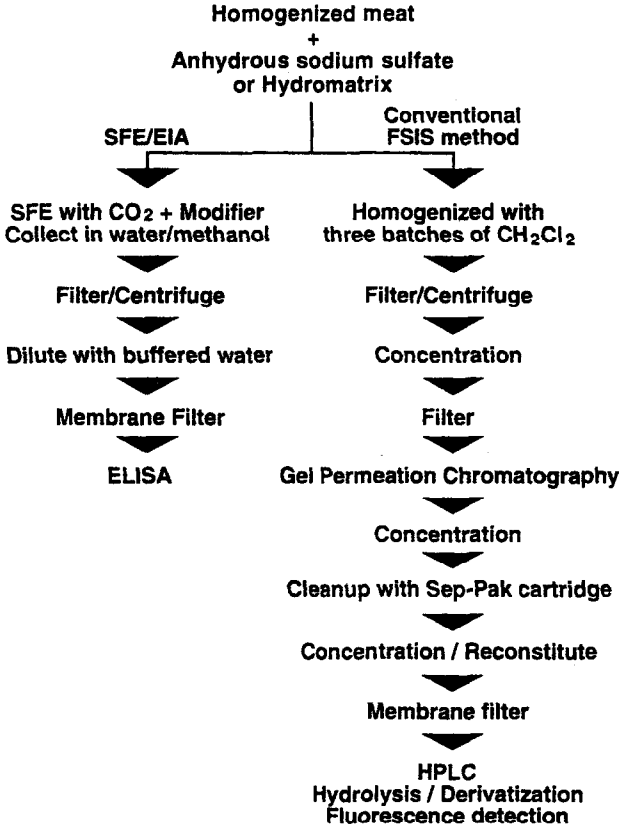

Figure 11 Analytical methodologies for the determination of carbamate pesticides in meats. (From Ref. 35.)

in combination with various forms of chromatography or spectroscopy. Most of these coupled or tandem techniques make use of on-line SFE rather than the off-line mode of extraction. An exception to this is the coupling of enzyme immunoassay (EIA) with SFE for both the qualitative and quantitative determination of toxicants in environmental and food samples. Figure 11 shows in a stepwise fashion the developed SFE-EIA method. This method employed only benign agents such as CO_2 and water and two simple filtration steps before applying the EIA for the quantitative determination of carbamate pesticides in meat products. This method is much simpler than the official method used by a regulatory agency, which is very solvent extensive, requires multiple clean-up steps, and a complex HPLC postcolumn derivatization method for determining the presence of carbamate pesticides in meats [52].

Another example from our method development studies that nicely illustrates the benefits of using either SFE or SFC is in the analysis of total fatty acid content in an industrial soapstock sample. In this case, a SFE method was developed that coupled both the extraction of the fatty

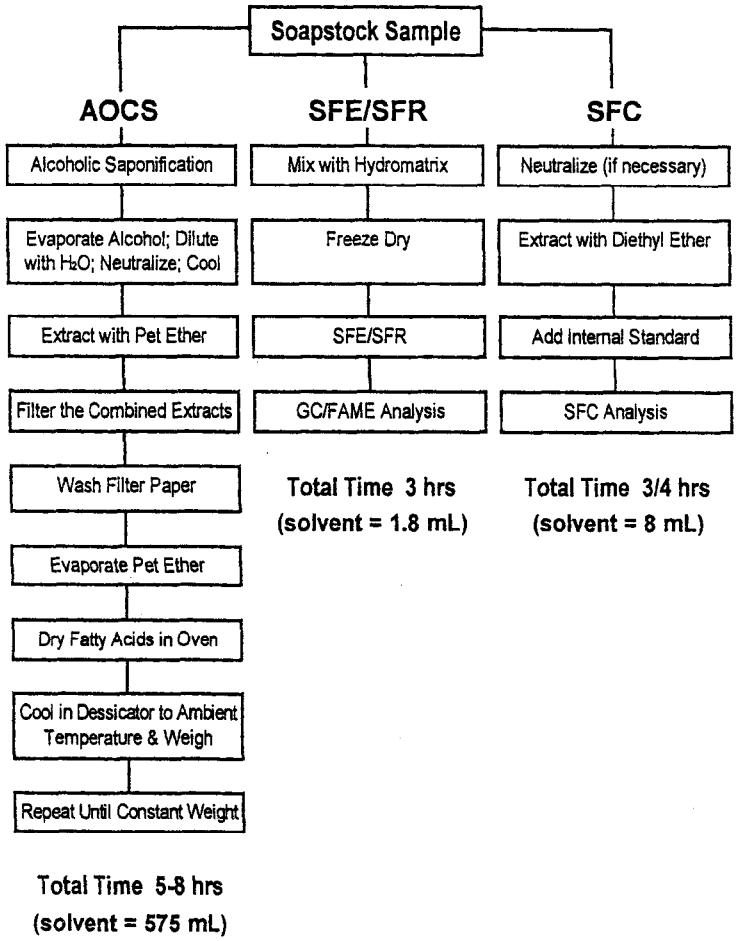

Figure 12 Comparison of AOCS Official Method G3-53 with the SFE coupled with an enzymatic-catalyzed reaction (SFE/SFR) and SFC methods. (From Ref. 53.)

acid moieties and triglycerides with a lipase-based methylation of the fatty acids to allow on-line analysis via gas chromatography [53]. This rapid method consisted of mixing the sample with the previously mentioned Hydromatrix, quickly freeze-drying this mixture, and then extracting and derivatizing the extract simultaneously using the coupled-automated SFE method. The benefits of such a technique are illustrated in Fig. 12, where the SFE/SFR (supercritical fluid reaction) method is contrasted with the conventional solvent and labor-intensive method, the American Oil Chemical Society (AOCS) Official Method G3-53, in a flow chart of the analysis. Note that the AOCS method consists of many manual steps, takes 5–8 hours depending on the analyst, and requires over 0.5 L of organic solvent. However, the SFE-based method takes only 3 hours and utilizes less than 2 mL of solvent. An alternative method, which is quite rapid but gives slightly lower results than either the AOCS or the SFE/SFR method, incorporates capillary SFC to analyze the soapstock sample. As indicated in Fig. 12, this method takes only 45 minutes and uses only 8 mL of solvent. Considering that the developed assays were applicable to monitoring tanker delivery trucks upon which time-based demurrage was being charged, the SFC method, even with its inherent inaccuracy, may be the preferred method, particularly if it can be used in a diagnostic fashion for detecting problematic shipment lots of this particular industrial by-product.

V. THE CONVERGENCE OF ANALYTICAL WITH PROCESSING SUPERCRITICAL FLUID METHODOLOGY

With the beginning of the new millennium it is important to assess the role of analytical SFE and SFC in terms of their future. Both techniques will continue to find niche analytical uses, probably SFE more than SFC, according to Smith [46]. From this author's perspective, SFC has entered an age in which standardization of methods and routine use of the technique are critical. Considering the limited number of commercial vendors for SFC, it is hoped that its use in chiral separation technology and perhaps SFC-MS (mass spectrometry) couplings will assure its continual use. Analytical SFC can be used as a template for the design and optimization of process SFC–based separations. SFC also competes favorably with normal phase liquid chromatography (LC) methods in terms of solvent reduction and column efficiency. Recently its use has been expanded in the simulated moving bed approach [54], while Taylor and King [55] have shown that coupling SFE with SFC in the preparative mode can provide greater fractionation and enrichment of targeted solutes than that achieved with supercritical fluid–based fractionation columns.

Automation in analytical SFE to date has been achieved using sequential analysis of multiple samples as typified by operation of the Isco Model 3560 extractor (Isco, Inc., Lincoln, NE). Such a unit can extract and collect 24 samples consecutively and is microprocessor controlled. The latter feature permits not only routine analysis, but the optimization of method development with respect to variables such as pressure, temperature, extraction time (fluid volume), cosolvent addition, and different sample types. This mode of operation can be viewed as an extension of combinatorial technology in which a wide variety of conditions, sample types, and phenomena can be rapidly studied and assessed. Currently this approach is being used in the author's laboratory for optimizing various processing applications of supercritical fluid technology as indicated in Table 8 [56]. The savings in time, labor, and reagent expense can be considerable using the above approach prior to scaling up a process. It is interesting to note in Table 8 that many of the processes investigated involve surveying reaction chemistry possibilities in critical fluid media. Reaction chemistry has been gradually integrated into analytical SFE, as the survey by

Table 8 Examples of Analytical Instrumentation Utilized in Nonanalytical Applications Involving Critical Fluids

Sterol ester fractionation using sorbents
SFE and methylation of phospholipids and steryl esters
Evaluation of enzyme catalytic activity in SC-CO_2
Optimization of SFE of cedarwood oil in SC-CO_2 and LCO_2
Sorbent selection for preparative SFC of phospholipids
SFE/SFC for enrichment of steryl esters from corn bran
SC-CO_2 extraction of pheromone components from fir needles
Selective extraction of components from RBO deodorizer distillate
Optimization of enzymatic hydrolysis of fat-soluble vitamins
Feasibility of enzyme-initiated acetylation of cedrol

Field [57] indicates, and this will be a continuing trend in the author's opinion, since reaction rates are pressure dependent and kinetic processes accelerated in supercritical fluids relative to those conducted in the condensed liquid state.

In summary, it has been the author's privilege to be at the inception of analytical SFC and SFE and to participate in their development over the past 30 years. In addition, his experiences and research in this field have involved not just the analytical use of supercritical fluids in the separation sciences, but also their application in the fields of chemical engineering, food technology, and natural products processing. There are differences in approach when using the separation sciences in engineering versus analytical chemistry, but the seminal principles behind techniques such as SFE and SFC are the same regardless of the area of application. Consequently, it is his feeling and philosophy that there is much to be gained by following developments on a multidisplinary front, and even more so in applying such knowledge in a synergestic fashion across several disciplines as the need arises.

REFERENCES

1. HS Booth, RM Bidwell. Solubility measurement in the critical region. Chem Rev 44:477–513, 1949.
2. BH Sage, WN Lacey. Volumetric and Phase Behavior of Hydrocarbons. Palo Alto, CA: Stanford University Press, 1939.
3. JC Giddings, MN Myers, JW King. Dense gas chromatography at pressures to 2000 atmospheres. J Chromatogr Sci 7:276–283, 1969.
4. MN Myers, JC Giddings. Ultra-high-pressure gas chromatography in micro columns to 2000 atmospheres. Sep Sci 1:761–776, 1966.
5. PA Peaden, JC Fjeldsted, ML Lee, SR Springston, M Novotny. Instrumental aspects of capillary supercritical fluid chromatography. Anal Chem 54:1090–1093, 1982.
6. JC Giddings, MN Myers, L McLaren, RA Keller. High pressure gas chromatography of non-volatile species. Science 162:67–73, 1968.
7. JW King. Fundamentals and applications of supercritical fluid extraction in chromatographic science. J Chromatogr Sci 27:355–364, 1989.
8. JW King, JP Friedrich. Quantitative correlations between solute molecular structure and solubility in supercritical fluids. J Chromatogr 517:449–458, 1990.
9. SR Allada. Solubility parameters of supercritical fluids. Ind Eng Chem Proc Des Dev 23:344–348, 1984.

10. Y Ikushima, T Goto, M Arai. Modified solubility parameter as an index to correlate the solubility in supercritical fluids. Bull Chem Soc Jpn 60:4145–4147, 1987.
11. JW King. Supercritical fluid adsorption at the gas-solid interface. In: TG Squires, ME Paulaitis eds. Supercritical Fluids—Chemical and Engineering Principles and Applications, ACS Symposium Series #329. Washington, DC: American Chemical Society, 1987, pp. 150–171.
12. E. Klesper. Chromatographie mit überkritischen Fluidenphasen. Angew Chem 90:785–793, 1978.
13. CF Poole, JW Oudsema, TA Dean, SK Poole. Stationary phases for packed column supercritical fluid chromatography. In: B Wenclawiak, ed. Analysis with Supercritical Fluids: Extraction and Chromatography. Berlin: Springer-Verlag, 1992, pp. 116–133.
14. TL Chester. Chromatography from the mobile phase perspective. Anal Chem 69:165A–169A, 1997.
15. Y Shen, ML Lee. Solvating gas chromatography using packed columns. In: PR Brown, E Grushka, eds. Advances in Chromatography, Vol. 38. New York: Marcel Dekker, 1998, pp. 75–113.
16. ST Sie, GWA Rijnders. Chromatography with supercritical fluids. Anal Chim Acta 38:31–44, 1967.
17. JW King, SE Abel, SL Taylor. SEC for sample cleanup using supercritical fluids. Proceedings of the 5th International Symposium on Supercritical Fluid Chromatography and Extraction, Baltimore MD, 1994, p. D-24.
18. ST Lee, SV Olesik, SM Fields. Applications of reversed-phase high performance liquid chromatography using enhanced-fluidity liquid mobile phases. J Microcol (Sep. 7):478–483, 1995.
19. ML Lee, K Markides, eds. Analytical Supercritical Fluid Chromatography and Extraction. Provo, UT: Chromatography Conferences, Inc., 1990.
20. TA Berger. Packed Column SFC. Cambridge, U.K.: Royal Society of Chemistry, 1995.
21. K Anton, C Berger, eds. Supercritical Fluid Chromatography with Packed Columns. New York: Marcel Dekker, 1998.
22. DR Gere. Supercritical fluid chromatography. Science 222:253–259, 1983.
23. LT Taylor. Trends in supercritical fluid chromatography: 1997. J Chromatogr Sci 35:364–382, 1997.
24. C Borch-Jensen, J Mollerup. Applications of supercritical fluid chromatography to food and natural products. Sem Food Anal 1:101–116, 1996.
25. JW King, JM Snyder. Supercritical fluid chromatography—a shortcut in lipid analysis. In: R McDonald, M Mossoba, eds. New Techniques and Applications in Lipid Analysis. Champaign, IL: American Oil Chemical Society Press, 1997, pp. 139–162.
26. KW Phinney. SFC of drug enantiomers. Anal Chem 72:204A–211A, 2000.
27. JW King. Applications of capillary supercritical fluid chromatography-supercritical fluid extraction to natural products. J Chromatogr Sci 28:9–14, 1990.
28. KD Bartle, AA Clifford, SA Jafar, JP Kithinji, GF Shilstone. Use of chromatographic retention measurements to obtain solubilities in a liquid or supercritical fluid mobile phase. J Chromatogr 517: 459–476, 1990.
29. MA McHugh, VJ Krukonis. Supercritical Fluid Extraction, 2nd ed. Boston: Butterworth-Heinemann, 1994, p. 368.
30. MEP McNally, JR Wheeler. Increasing extraction efficiency in supercritical fluid extraction from complex matrices. J Chromatogr 447:53–63, 1988.
31. SL Taylor, JW King, L Montanari, P Fantozzi, MA Blanco. Enrichment and fractionation of phospholipid concentrates by supercritical fluid extraction and chromatography. Ital J Food Sci 12:65–76, 2000.
32. M Saito, Y Yamauci. Isolation of tocopherols from wheat germ oil by recycle semi-preparative supercritical fluid chromatography. J Chromatogr 505:257–271, 1990.
33. P Lembke. Production of high purity n-3 fatty acid-ethyl esters by process scale supercritical fluid chromatography. In: K Anton, C Berger, eds. Supercritical Fluid Chromatography with Packed Columns. New York: Marcel Dekker, 1998, pp. 429–443.
34. E Stahl, KW Quirin, D Gerard. Dense Gases for Extraction and Refining. New York: Springer-Verlag, 1988.
35. JW King. Analytical supercritical fluid techniques and methodology: conceptualization and reduction to practice. J Assoc Off Chem Int 81:9–17, 1998.

36. SB Hawthorne, JW King. Principles and practice of analytical SFE. In: M Caude, D Thiebaut, eds. Practical Supercritical Fluid Chromatography and Extraction. Chur, Swizerland: Harwood Academic Publishers, 1999, pp. 219–282.
37. HJ Vandenberg, AA Clifford. Polymers and polymer additives. In: AJ Handley, ed. Extraction Methods in Organic Analysis. Sheffield, England: Sheffield Academic Press, 1999, pp. 221–242.
38. AAM Stolker, MA Sipoli Marques, PW Zoontjes, LA Van Ginkel, RJ Maxwell. Supercritical fluid extraction of residues of veterinary drugs and growth-promoting agents from food and biological matrices. Sem Food Anal 1:117–132, 1996.
39. L. McDaniel, GL Long, LT Taylor. Statistical analysis of liquid trapping efficiencies of fat soluble vitamins following SFE. J High Resolut Chromatogr 21:245–251, 1998.
40. ML Hopper, JW King, JH Johnson, AA Serino, RJ Butler. Supercritical fluid extraction (SFE): multivessel extraction of food items in the FDA total diet study (TDS). J Assoc Off Anal Chem Int 78:1072–1079, 1995.
41. WN Moore, LT Taylor. Analytical inverse SFE of polar pharmaceutical compounds from cream and ointment matrices. J Pharm Biomed Anal 12:1227–1232, 1994.
42. JW King, Z Zhang. Selective extraction of pesticides from lipid-containing matrices using supercritical binary gas mixtures. Anal Chem 70:1431–1436, 1998.
43. LT Taylor. Supercritical Fluid Extraction. New York: John Wiley, 1996.
44. MD Luque de Castro, M. Valcarel, MT Tena. Analytical Supercritical Fluid Extraction. Berlin: Springer-Verlag, 1994.
45. JR Dean, ed. Application of Supercritical Fluids in Industrial Analysis. London: Blackie Academic, 1993.
46. RM Smith. Supercritical fluids in separation science—the dream, the reality and the future. J Chromatogr A 856:83–115, 1999.
47. SJ Lehotay. Supercritical fluid extraction of pesticides in foods. J Chromatogr A 785:289–312, 1997.
48. JW King. Critical fluids for oil extraction. In: PJ Wan, PJ Wakelyn, eds. Technology and Solvents for Extracting Oilseeds and Nonpetroleum Oils. Champaign, IL: AOCS Press, 1997, pp. 283–310.
49. JM Snyder, JW King, MA Jackson. Fat content for nutritional labeling by supercritical fluid extraction and an on-line lipase catalyzed reaction. J Chromatogr 750:201–207, 1996.
50. Oil in oilseeds: supercritical fluid extraction method—AOCS Official Method Am 3-96. Official Methods of American Oil Chemical Society. Champiagn, IL: AOCS Press, 1997.
51. JM Snyder, JW King, Z Zhang. Comparison of volatile analysis of lipid-containing and meat matrices by solid phase micro- and supercritical fluid-extraction. In: CJ Mussinan, MJ Morello, eds. Flavor Analysis: Developments in Isolation and Characterization. ACS Symposium Series #705. Washington, DC: American Chemical Society, 1998, pp. 107–115.
52. K. Nam, JW King. Supercritical fluid extraction and enzyme immunoassay for pesticide detection in meat products. J Agric Food Chem 42:1469–1474, 1994.
53. JW King, SL Taylor, JM Snyder, RL Holliday. Total fatty acid analysis of vegetable oil soapstocks by supercritical fluid extraction/reaction (SFE/SFR). J Am Oil Chem Soc 75:1291–1295, 1998.
54. T Giese, M Johanssen, G Brunner. Separation of stereoisomers in a SMB-SFC plant: determination of isotherms and simulation. Proceeding of the International Meeting of the GVC-Fachausschuss Hochdruckverfahrenstechnik, Karlsruhe, Germany, 1999, pp. 275–278.
55. SL Taylor, JW King. Optimization of the extraction and fractionation of corn bran oil using analytical supercritical fluid instrumentation. J Chromatogr Sci 38:91–94, 2000.
56. JW King, FJ Eller, SL Taylor, AL Neese. Utilization of analytical critical fluid instrumentation in non-analytical applications. Proceedings of the 5th International Symposium on Supercritical Fluids, Atlanta, GA, 2000, pp. 1–20.
57. J Field. Coupling chemical derivatization reactions with supercritical fluid extraction. J Chromatogr A 785:239–249, 1997.